THE NEWALA EPIDEMIC

III. THE VIRUS: ISOLATION, PATHOGENIC PROPERTIES AND RELATIONSHIP TO THE EPIDEMIC

By R. W. ROSS*

From the Virus Research Institute, Entebbe

CONTENTS

I. INTRODUCTION

This report on a large outbreak of disease, known locally as 'Chikungunya', in the Newala district of Tanganyika concerns the circumstances of isolation of strains of virus, some of their properties, and their relation to the epidemic. The work, reported here, started in Newala from 18 February to 10 March, 1953, and continued thereafter in Entebbe.

II. DESCRIPTION OF CASES SEEN

The previous paper in this series (Lumsden, 1955) discusses the epidemic as a whole, but an outline of the position in late February and early March is relevant. A disease believed to be new to the area had attacked a high proportion of the

* Now of the Department of Pathology, University of Cambridge.

Africans of all ages resident on the Makonde plateau; it was sufficiently distinctive to warrant the coining of a new descriptive name. The non-occurrence of second attacks had been widely attested. At this stage only occasional cases were occurring in widely scattered places. Poor communications and the primitive social structure of the community made it necessary to go on foot to inquire for fresh cases from house to house in those areas where sickness was vaguely reported.

A number of the features of the illness made this easier than was foreseen. First, the novelty of the disease, which was dramatic, with the very sharp onset of crippling joint pains, severe fever, and eventually the conspicuous rash. Secondly, the sequelae were always minor and so the disease was not feared. Thirdly, malaria was very uncommon in the district and readily distinguishable clinically. Clinical findings of a large number of cases observed throughout their course have already been described (Robinson, 1955), but an account of the condition of the cases seen at this period will be given. Of these, thirty were first visited within a few days of the start of symptoms. All had malaise of sudden onset, and joint pains were reported by most. Oral temperatures varied between 98·4 and 104·9° F. Table 1 gives the distribution of temperatures recorded. The five apyrexial patients

Table 1

Below 98·9° F.	99·0–99·9° F.	100·0–100·9° F.	101·0–101·9° F.	102·0–102·9° F.	103·0–103·9° F.	104·0–104·9° F.
5	4	4	3	5	5	4

complained of typical symptoms and one of them had a rash, another was in a shocked condition, and the three others were in houses in which typical febrile cases were also present. The more severe cases lay on their sides with all their joints in flexion, a position which gave a slight relief from pain. Many covered their heads with a cloth, a local sleeping custom, which in daylight suggested mild photophobia. The extension of an arm was in some a most painful process, in one case (donor of serum 36) only done in little jerks. Painful eye movements were not recorded, and complaints of headaches were slight and infrequent. Little was seen of the course of particular illnesses as most patients were seen on two occasions only. Seven patients were seen with rashes, one 3 hr. after onset (donor of serum 19) and the others $1\frac{1}{4}$, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3 and 7 days after onset.

Cases of several days' duration and convalescents were seen in much greater numbers and reported that the disability generally lasted for about a week. A few remained ambulant throughout, but one man was in bed for 3 weeks. Occasional individuals complained of joint pains for months. In general, the course of the illness at this time was milder than earlier in the epidemic. However, the general impression formed was of a single type of illness of varying degrees of severity.

III. MATERIALS AND METHODS

(1) *General*

All the available information about the epidemic pointed to its similarity to dengue in explosiveness, in probable association with an insect vector, and in clinical manifestations. This markedly influenced the methods used to investigate

it, and resulted in the use of young mice (Meiklejohn, England & Lennette, 1952; Sabin, 1952; Schlesinger & Frankel, 1952). Newala district was very far from Entebbe and without local laboratory facilities, so all materials had to be brought to it. Because of the importance of speed, all staff and materials were flown to Lindi and then taken by road to Newala, and this severely limited the weight that could be taken. The other workers with dengue virus had all experienced initial difficulties with isolation. It was consequently thought that success would be most probable if human sera and blood-sucking insects were collected from as many places as possible.

(2) *Laboratory animals*

The mice used were Albino Swiss from the Entebbe Institute colony. The original batch were 17 days old on departure and were weaned on that day. Standard mouse boxes designed for a group of six adults were partitioned into two, and eight distinguishably marked mice in two groups of four were put into each side. Fresh mice were brought on 28 February 1953, some being young adults and the rest pregnant females which produced litters in Newala. On the journey mice were fed on bread and milk; 'Farex' baby food was used after arriving in Tanganyika because a large consignment had been condemned as unfit for human consumption. The health of these mice was good before inoculation, except for a few boxes in which heat and fermentation of the mouse cubes in the bedding killed all the occupants during the road journey to Newala. Two young rhesus monkeys were also taken in a duralumin fish box fitted with a wire front.

The work was greatly handicapped by the high death-rate of the mice of the colony. Cannibalism, particularly of young litters by their mothers, was very frequently observed. In addition, *Salmonella typhi-murium** was periodically cultured from mice and spontaneous paralysis of the hind limbs was regularly observed; a number of strains of Theiler's mouse encephalomyelitis have been obtained by the passage of filtered brain material from such animals both before and after the work reported here. However, the main cause of this high mortality was neither of these agents. Deaths were scattered fairly evenly over the third and fourth weeks of life, were most frequently in ones and twos and were usually several days apart. External signs of illness were confined to sluggishness and fluffing of the fur and was usually limited to 2 days or less. Dead animals usually lay on their ventral surface, without extension of the limbs or head retraction or the sharp angulation of the spine usually seen in experimental encephalitis in mice. Many attempts at discovering the cause of death by post-mortem examinations, cultures of selected organs or the subinoculation of tissues were not successful. Consequently non-specific deaths cannot be excluded from consideration in the work that follows.

(3) *Laboratory equipment*

The major items were a small pressure cooker which was heated on a primus stove, and a hand centrifuge. Mosquitoes were ground in sterile Pyrex tubes with glass rods and glass powder. Normal saline was, however, used as diluent, because

* The identity of a typical strain was confirmed by Dr Joan Taylor, of the Salmonella Reference Laboratory, Colindale.

filtered bovine albumen had grown moulds. All inoculations were done with ¼ ml. tuberculin syringes. Blood samples were taken in 30 ml. vacuum venules.

(4) *Inoculations*

Attempts to isolate virus were made with the following material: human sera from early acute cases, mosquitoes caught in areas where fresh infections were reported, bed bugs from a hut in which a patient was living, and artificially reared mosquitoes which had been induced to feed on febrile patients in Newala. The detailed results with each of these kinds of source material and the results observed are reported below.

(*a*) *Human sera*

As the main object of this work was to isolate the virus responsible for the outbreak, the greatest emphasis was placed on the examination of acute sera. Repeat bleedings were not performed except for serology. Cases with the features already described were bled from the arm in their own homes. The needle was then cut from the venule, the contained blood was allowed to clot, and retraction was aided by shaking it free from the tube walls. The numbered venule was then put into a thermos bottle with ice. All such sera collected in the course of the day's visits were centrifuged on return to Newala. 0·25 ml. from each tube was drawn into a sterile syringe through a 20-gauge needle, which pierced the rubber cap of the venule after cleaning with ether. Four similarly marked mice were inoculated intracerebrally and the rest of the serum was inoculated intraperitoneally into rhesus monkey MR 1058, and the venule was then replaced in ice-water. Twelve such groups were inoculated at Newala before 28 February 1953, on which date these and the monkey were returned to Entebbe and fresh mice were brought, seventeen more groups of which were inoculated with sera before 10 March 1953. In addition to isolation attempts at Newala, eight passages were done with the brains of mice which had sickened after intracerebral inoculation of serum. Aliquots from some of the acute sera which arrived in Entebbe on 1 March 1953 were pooled and inoculated into monkey MR 1015 on 9 March 1953, 0·5 ml. intracerebrally, 0·75 ml. intraperitoneally, and 0·75 ml. subcutaneously. A very small sample of pre-inoculation serum was taken. Mice died after the inoculation of twenty-nine sera, twenty of which were inoculated at both Newala and Entebbe and nine at Entebbe only.

(*b*) *Captive mosquitoes allowed to feed on patients*

Forty laboratory-bred *Aedes aegypti* were taken to Newala in individually numbered vials for feeding on febrile patients, to give further chances of obtaining virus. Twenty-eight mosquitoes returned alive, five of which contained a lethal agent which was repeatedly passaged; twelve more killed mice on inoculation but passage was not attempted; one was rapidly toxic on inoculation; nine showed no sign of virus on passage; and one died on the way back. A further nine Newala *A. aegypti* were bred out, used as replacements, and fed in the same way. Two of them contained an agent which was passed on several occasions; five probably

contained such an agent and two were inoculated into adult mice which survived. Numerous attempts to demonstrate the transmission of the agent by the bite of these captive mosquitoes on baby mice failed, and the last survivor bit a human volunteer without visible effect and was inoculated into baby mice without killing them.

(*c*) *Wild mosquitoes*

The details of mosquito collection and classification have already been described (Lumsden, 1955). Each batch, comprising all the members of a genus caught on one day, was ground in saline, centrifuged and inoculated into mice. A 0·25 ml. aliquot of the same material was also inoculated into monkey MR 1067 intraperitoneally. Seventeen batches were inoculated in Newala and another one in Entebbe after storage on ice. Deaths occurred in sixteen out of the eighteen groups of mice inoculated. The two groups in which all survived consisted of adult mice previously inoculated with batches of *A. aegypti*. This part of the work was less informative than was hoped because mice died following inoculation with members of each species of mosquitoes, some members of which were thought recently to have bitten humans and thus picked up the agent. The portions of these suspensions not used at once were pooled in 50% glycerine and stored in ice-water until their return to Entebbe, where eight such pools of glycerinated mosquitoes, diluted tenfold, were inoculated into young mice. Two of the three *A. aegypti* pools yielded virus, one of them on repeated occasions after storage for up to 8 weeks at 4° C.; the third killed all mice within 24 hr. of inoculation and was classed as toxic. One *Culex* pool yielded virus and two were toxic. The *Anopheles* pool, containing fewer insects, killed no mice within 30 days of inoculation.

(*d*) *Bed bugs*

Bed bugs from the bedsteads of individuals recently suffering from the disease were brought to Entebbe alive. Further batches were stored in 50% glycerine and refrigerated. Seventeen fresh bugs were inoculated individually into groups of mice. Deaths occurred in five of the groups. Three bugs were confirmed as infective by repeated brain-to-brain passages. Two out of the three pools of bed bugs glycerinated at Newala killed mice on arrival at Entebbe. A series of transmission attempts were made by feeding wild bed bugs on baby mice, but no agent was recovered from the few mice which died.

IV. RESULTS

(1) *Response of mice to inoculation*

The occurrence of a large mortality in inoculated mice had not been expected, as workers with dengue virus experienced difficulty in infecting mice with it and transmitting it in series. Moreover, the stability of any agent or agents was unknown. For this reason, as mice sickened or died the subinoculation of emulsions of various of their organs was continued until a regular and predictable passage procedure was evolved. As seventy-nine samples of six different kinds of source material killed mice on inoculation, the use of this method entailed the

simultaneous presence in the animal room of mice subinoculated with infected tissues from many sources and coming from a colony in which lethal agents were known to be present.

Although non-specific causes of death were known to be present among the mice, most of the deaths amongst the adults were probably due to their inocula. This is suggested by the high mortality of those inoculated with sera and the low mortality of those inoculated at the same time with bed bugs. A comparison of the numbers surviving in the four following groups, which comprise all the young adult mice inoculated intracerebrally in the initial period with materials collected at Newala, makes this suggestion clear.

(*a*) Of all the mice receiving acute human sera fifty-six survived out of the 164 inoculated, i.e. 34·1 %.

(*b*) Thirty-nine mice out of the sixty-four inoculated with captive mosquitoes survived, i.e. 60 %.

(*c*) Thirty-four mice survived out of the eighty-three inoculated with pools of wild mosquitoes, i.e. 40·9 %.

(*d*) Seventy-six mice survived out of the eighty-three inoculated with bed bugs, i.e. 91·5 %.

Although the death-rate in mice inoculated in Newala was partly attributable to the stress of a long and hot journey, it corresponded fairly closely with that seen subsequently in the mice inoculated in Entebbe with the same materials. The results of certain of these primary inoculations in baby mice are of special interest. Table 2 gives the days of death of those mice from groups in which two or less in any one group survived intracerebral inoculation. The sources marked † were inoculated into duplicated groups of mice after the material had been diluted fivefold. Of the twenty-four sources listed seventeen killed mice, so that deaths were spread over 4 days or less; of these seventeen, eleven consisted of mice, inoculated with sera numbers 19, 22, 24, 27, 30 and 103, mosquitoes M15, M33 M41, M42 and *Culex* pool 3, which had a median survival period of 4 days or less; the remaining six consisted of mice inoculated with sera numbers 23, 45, 50 and mosquitoes M8, M29, M35, which had a median survival period of about 9 days. The data suggest the presence of two agents, one with a short incubation period and one with a longer; however, their differentiation by incubation period was prevented by the presence of a small amount of antibody in some of the acute sera, which had the effect of prolonging the illness of some of the mice infected with the more rapidly lethal virus (Table 5).

The significance of subinoculations in the initial period was reduced by the number of passages being done at any one time, each increasing the possibility of accidental contamination or confusion, and by the presence of accidental infection among the mice themselves. However, four points of subsequent value were established. First, 6-day-old mice were more readily infected than weaned mice. Secondly, passage by the intracerebral inoculation of brain material was more frequently successful in causing infection than either liver or spleen. The passage of even brain material was uncertain and regularity was increased by diluting the inoculum, suggesting that auto-interference occurred on occasion.

Table 2

(A) *The results of inoculating suckling mice with certain human sera*

Serum no.	Name	Place	Intracerebral: Survival ratios	Intracerebral: Days on which deaths occurred	Intraperitoneal: Survival ratios	Intraperitoneal: Days of death
*S 19	Twikali d/o Mwachi	Namikupa	0/5	3, 3, 3, 3, 4	1/5	4, 4, 5, 8
S 20	Fatu d/o Nangororo	Namikupa	0/5	2, 3, 5, 7, 14	4/5	25
S 21	Josephine d/o Mniwasa	Tandahimba	0/4	2, 2, 3, 13	5/5	—
S 22	Stella d/o Mbepo	Tandahimba	1/5	3, 3, 5, 5	3/4	5
*S 23	Joi d/o Mbepo	Tandahimba	1/4	6, 6, 7	5/5	—
S 24	Nandipache d/o Nancumbe	Mkanjawana	0/3	2, 4, 4	2/4	5, 5
S 27	Joi d/o Athumani	Liteho	0/4	3, 4, 4, 4	3/4	7
*S 30	Cypriani s/o Chionekwa	Tandahimba	0/4	3, 5, 5, 6	4/4	—
*S 45	Chivemili s/o Masudi	Kitama	0/5	8, 8, 9, 10, 10	—	—
†S 46	Esha d/o Asmani	Kitama	2/8	3, 5, 6, 8, 9, 10	—	—
†S 50	Samuli s/o Samuli	Nanyamba	3/8	7, 8, 9, 10, 10	—	—
†S 103	Ahamadi s/o Hamisi	Kitama	0/8	2, 2, 2, 3, 3, 4, 4, 6	—	—

(B) *The results of inoculating infected captive mosquitoes into baby mice*

Mosquito no.	No. of serum fed on	No. of days survived	Temperature of host	S/R	Days of death
M 5	S 26	24	103°	0/5	6, 6, 7, 7, 20
M 8	—	20	102°	0/6	7, 7, 7, 7, 7, 7
M 14	S 30	36	101°	0/5	6, 12, 13, 14, 14
M 15	S 30	19	101°	0/5	3, 3, 3, 3, 3
M 29	S 36	20	104·9°	0/5	7, 8, 8, 8, 8
M 33	S 27	15	101·0°	0/4	2, 5, 5, 5
M 35	S 36	21	104·9°	0/5	6, 7, 7, 7, 7
M 41	S 36, S 37	33	—	0/5	3, 3, 3, 4, 4
M 42	S 36	33	104·9°	0/5	2, 4, 5, 5, 5
M 50	S 36	20	104·9°	0/5	2, 4, 4, 4, 7

(C) *The results of inoculating wild mosquito pools into baby mice*

	S/R	Days of death
†*Aedes* pool 1	1/8	4, 4, 4, 5, 5, 9, 10
†*Culex* pool 3	0/7	2, 2, 2, 2, 2, 3, 3

* Chikungunya virus re-isolated after 2½ years. Storage at −20° C.

† Materials marked thus inoculated into two litters of suckling mice after tenfold dilution.

(2) *Behaviour of agents on serial passage*

The preceding section has emphasized the possibilities of cross-contamination inherent in the work done before the stability of the agents recovered became known, and the value of dilution passage was demonstrated. Thereafter most of the agents recovered were stored at $-20°$ C. in glycerine. To eliminate cross-infections return was made to material which had already killed mice on the original inoculation and which had been stored in the refrigerator in the interval. The first short incubation strain re-examined for this reason came from acute serum S 27 which had been stored for 43 days at $-20°$ C. This killed four out of the four 6-day-old mice inoculated with it, and four passages were completed by the fifteenth day. Another strain re-examined came from pool I of wild *A. aegypti* glycerinated in Newala and stored for 47 days at about 4° C. This killed five out of the ten 7-day-old mice inoculated with a 1/5 dilution; subinoculation proved regularly lethal to baby mice and five passages were completed in 10 days. The suspension of the body of a captive mosquito, M 42, which had survived an infective feed for 36 days, killed five out of the five 6-day-old mice inoculated and five passages were completed in 15 days. A strain apparently exactly similar was derived from S 32, but some of the passages with this strain had been done at a time when there were very many chances of cross-infection.

(3) *Evidence that the agents were viruses*

Each of these four strains was an agent lethal for baby mice, could pass through a Seitz filter, and could be passaged apparently indefinitely in dilute brain suspensions with the regular production of characteristic symptoms in the infected animals, often on the second day. These animals, when disturbed early in the infection, fell on their sides, extended their limbs and heads fully, and exhibited a coarse spasmodic tremor. As the infection progressed fits of this nature became more frequent, and within 24 hr. the animals died in a typical attitude; some strains killed within 18 hr. Filtrates which produced such symptoms on inoculation were cultured on numerous bacteriological culture media without producing any growth, and the microscopical examination of them showed no visible particles. The addition of penicillin, aureomycin, terramycin or streptomycin had no effect on the infectivity of the filtrates. These points, together with resistance to glycerine and infectivity in high dilution of mouse brain, confirm that the agents are viruses.

The similarity of the four strains described is shown in the following observations. Infectivity titrations were done in 6- and 28-day-old mice with stored suspensions of mouse brain. The infectivity titres in 6-day-old mice varied between $10^{-6·8}$ and 10^{-8}, with the earliest deaths occurring on the second day and the median death on the fourth day; occasional deaths only occurred in 28-day-olds. An antiserum prepared against the S 27 strain neutralized nearly 10,000 LD_{50}'s of all four of these strains and failed to neutralize 'long incubation' strains such as derived from M 29. The name Chikungunya virus was applied to the four strains described and to others with similar properties.

The name Makonde virus was applied to strains such as M29, which had a long incubation period in baby mice; work reported elsewhere shows that many strains of Makonde virus were present, were identical, and were readily distinguishable from Chikungunya virus. For example, twelve mice were immunized against the four strains of Chikungunya by repeated inoculations of virus. Ten days later the survivors were challenged with a lethal dose of Makonde virus, all died. Mice immunized with Makonde virus by the same method survived challenge. It has been shown previously that the incubation period only gives an indication of which of the two viruses was present; however, all strains which killed mice older than 14 days with regularity proved to contain Makonde when tested serologically.

Table 3. *The behaviour of four strains of Chikungunya virus*

Strain designation	S27	S32	M42	A.A. pool
Type of source material ...	Serum of Athumani	Serum of Nakula	Mosquito fed on Liza Lilas	Wild *Aedes aegypti*
Locality collected	Liteho	Muhidya	Makongonda	Newala dis.
Titre in 28-day-old mice, i.c.	1 in–4*	1 in–5*	2 in–4*	—
	1 in–6	1 in–7	1 in–7	—
			1 in–8	None
Mortality on inoculation of large i.p. dose	1/10	1/11	0/12	2/11
Titre in 6-day-old mice	8·0	8·0	7·5	6·8
Median day of occurrence of death	4·0	4·5	4·0	4·0
Dilution of serum of MR 1015 saving 50 % of mice after inoculation with 100 LD_{50}	1/320	1/320	1/320	1/300
Neutralization index of specific serum of rabbit immunized with Makonde R394 (NI–18)	0	0	0	0
Neutralization index of specific serum of rabbit immunized with Chikungunya R400 (S27)	4·1	3·8	4·0	4·2
Mortality ratio in mice immunized with the above strains and challenged with S30 virus, control mice below	12/12	12/12	12/12	9/9
	8/8	12/12	11/11	12/12

* 1 in–4 signifies 1 death in 10^{-4} dilution and so on.

(4) *The relationship of Chikungunya and Makonde viruses to strains of dengue*

Hyperimmune sera were prepared in mice against strains of Chikungunya, Makonde and Hawaiian and New Guinea 'B' dengue viruses. These, with some Sindbis antiserum (Taylor & Hurlbut, 1953), were tested in cross-neutralization tests. These tests were done using the technique described by Smithburn (1951), but 6-day-old mice were used for tests with Sindbis and Chikungunya virus. A relationship is suggested between Chikungunya and Hawaiian dengue virus, but no interrelationship between any of the others. The specificity of the antisera was probably reduced because of the multiple inoculations used in their

preparation. More work on the serological identification of these viruses will be reported elsewhere. Reciprocal cross-neutralization was also observed with Semliki Forest virus and this will be reported more fully with other serological work.

Table 4. *Neutralization indices of five antisera when mixed with five viruses*

	Antisera				
Viruses	Makonde	Chikungunya	Hawaiian	New Guinea 'B'	Sindbis
Makonde	2·6	0	0	0	0
Chikungunya	0	3·7	0	0	0
Hawaiian	0	1·6	2·2	0	0
Dengue New Guinea 'B' Dengue	0	0	0	2·0	0
Sindbis	0	0	0	0	3·0

V. CHIKUNGUNYA VIRUS

(1) *Behaviour in mice*

All strains classified as this virus were lethal to 6-day-old mice on intracerebral inoculation, with an initial incubation period often less than 48 hr. Continued serial passage of three such strains resulted in a gradual extension of incubation period until infected mice sickened on day 3 and died on days 4–7. Over 100 serial passages of one such strain failed to increase its virulence towards adult mice. To determine the age at which mice become resistant a series of litters of suckling mice aged from 6 to 20 days were inoculated intracerebrally with about 10^5 LD_{50}. All mice younger than 12 days died with typical symptoms, three out of five at 12 days old died and thirty-two out of the forty older than 12 days survived. However, in other experiments 13-day-old mice have been infected with material of very high titre. In titrations done in parallel in adult and baby mice, a few of the adults died amongst those inoculated with high dilutions. The first specific antiserum for this virus was prepared in adult mice which received a series of four intracerebral inoculations of stock diluted tenfold, at weekly intervals; the neutralization index of this with the homologous virus was 3·7.

(2) *Susceptibility of other animals*

100 LD_{50} were inoculated into each of six guinea-pigs, two subcutaneously, two intraperitoneally, and two intracerebrally. One animal of the intracerebral couple died with bacteraemia on the third day. Virus was not recovered from the brain of this, or from the blood of any of the others, which were bled on alternate days. Two rabbits received intraperitoneally the filtrate from two mouse brains infected with S 27 and suspended in 6 ml. of 10 % normal rabbit serum; one died the same day and the other on the third day. Virus was not recovered from the blood or brain of either. Two more rabbits were given 0·5 ml. of a similar filtrate mixed with 1·0 ml. of a Freund type of adjuvant by the same route, and these survived to give high-titre antisera.

VI. THE RELATIONSHIP OF THE VIRUS TO THE EPIDEMIC

(1) *The collection of sera for antibody studies*

Several patients who had been bled in the acute phase were bled again when convalescent. The number was small because cases were first found scattered over a wide area, some of them taking a long time to reach, because the work was done in rather a short period, and because the Makonde tribe as a rule seem to travel a great deal, so that convalescents were frequently far from their homes when rebleeding was attempted. In work of this kind the greatest stress must be put upon the serological testing of pairs of sera, because only by doing so can the development of a particular type of antibody be related to a definite illness. Fifteen pairs of sera were available, thirteen from donors whom the author had had an opportunity of examining in the acute stage, three having been taken by an African dresser. A further five pairs were from Africans loaned by the health department at Lindi to assist in the work, all of whom had been bled before leaving for Newala, and four of whom suffered from minor fevers whilst working there. A series of twelve sera were taken from the inhabitants of two villages, Lihombo and Makukwe, who were believed to be the earliest affected on the central plateau; a further series of sixteen were taken from schoolgirls in the Mission School at Newala who had been infected in December while under the care of Dr Robinson, and whose records were available. One sample was obtained from an Asian who had visited Newala for a period of only 3 hr. and had suffered a typical severe attack which began 3 days after his visit. This sample was taken 22 days after the onset. One further sample was obtained from a woman who had undergone a typical attack and was still suffering from severe joint pains. The sera listed in this section are those which arrived safely in Entebbe in suitable condition for testing; further sera were taken and were either lost through the breakage of tubes or were too heavily contaminated with bacteria. All the convalescent sera taken were kept at the circumambient temperature until their arrival at Entebbe and were much haemolysed.

(2) *Antibody studies with Chikungunya virus*

Because of the disadvantages of working solely with baby mice, antibody studies with this virus were postponed until its adaptation to adult mice had been unsuccessfully attempted. Three methods of adaptation were tried in succession: first, serial passage in baby mice with periodic virulence tests in 21-day-olds; secondly, passages in 21-day-old mice when ruffling and cyanosis of the tail was observed; and finally, alternate passages between 6- and 21-day-olds. The first method was continued for over 121 passages without any detectable effect; in the second the virus was lost after the fifth passage; and the final method was not completed. Blind passages were considered inadvisable once a lethal strain of Theiler's mouse virus had been detected in the mice of the colony.

(*a*) *Technique*

A neutralization test was developed using 6-day-old mice which allowed nine sera to be tested at a time. A series of $2\frac{1}{2} \times \frac{3}{8}$ in. tubes were labelled with

strips of sticking plaster, four for each serum to be tested, and four for each normal and immune serum, and arranged in a wire rack. 0·4 ml. of each serum to be tested was measured accurately into the first tube of each series into which an equal volume of fresh normal guinea-pig serum had been already placed; the contents of the tube were then well mixed and from it 0·2 ml. aliquots were transferred into the other three tubes. Dilutions of virus were made up in 10% normal rabbit serum containing approximately 0·3 LD_{50} up to 30,000 LD_{50} per 0·02 ml. volume in decimal steps; 0·1 ml. of the appropriate dilution was put into each serum tube, the four weakest going into the known normal serum and the four most concentrated into the others. After mixing and incubating for 2 hr. at 37° C. the tubes were transferred to an ice-bath and six baby mice were inoculated intracerebrally with the contents of each tube, 0·02 ml., to each mouse. The mice were then returned to their mothers and deaths were recorded up to the fourteenth day, when the survivors were counted; end-points were calculated by the method of Reed & Muench (1938). The neutralization index given for any serum was the difference between the logarithm of its end-point and that given by the control normal serum. In testing paired sera, both samples were tested at the same time. Two pairs of sera, both of which showed a very slight rise in N.I. in the second sample, were further tested by diluting each serum in each pair in twofold steps, adding complement and a final concentration of about 100 LD_{50} of virus to each tube, and inoculating mice in the way already described; a sharp rise of antibody was demonstrated in both serum pairs in this way and the results are listed as neutralization titres (N.T.) (Table 6).

(*b*) *Results*

Fifteen pairs of sera from cases (Table 5) were tested by this technique, and a rise of neutralization index of 1·3 or greater was observed in each case. In very few of the convalescent sera was an end-point reached.

The sera of the five African staff are of interest (Table 6). Paired sera were available from four of them and the early sample only from the fifth. Four of them worked as mosquito catchers where fresh cases of the disease had been reported and were therefore much exposed to infection; the fifth was a driver and was much less exposed. Kalindaya had a typical attack and his serum showed a rise in antibody by the standard technique. Mwambe and Fidelis had abortive illnesses, and a rise in antibody titre was demonstrated in their sera by a dilution technique. The driver Longole, who suffered from a very short bout of fever, was a chronic malarious subject; his serum showed no antibody either before or after this fever. Kipande remained well throughout, and the single serum sample taken from him before he left Lindi contained antibody. It is probable that he had been immunized before he left the coast, and it is possible that the attacks of Mwambe and Fidelis were abortive because of a previously acquired low-grade immunity.

All twelve sera from the early cases on the plateau (Table 7 (*a*)) neutralized 100 LD_{50} of virus or more, as did twelve of the sixteen sera from convalescent schoolgirls (Table 7 (*b*)) and the two other convalescents (Table 7 (*c*)). The sera of the three monkeys MR 1015, MR 1067 and MR 1058, all developed antibody to this

Table 5. *Neutralization indices of paired sera collected in Newala district*

Serum no.	Name	Days after onset	Date	Virus used	N.I.	Locality collected and remarks
12*a*	Egbert s/o	2	22. ii. 53	S 27–112	< 8	Newala Hospital, said
69	Hussein	11	5, iii. 53	S 27–112	> 2·8	to be typical
13*a*	Edna Tola	1	22. ii. 53	S 27–112	1·1	Newala Hospital, said
67		10	5. iii. 53	S 27–112	> 2·8	to be mild
15*va*	Saidi s/o Sulemani	1	25. ii. 53	S 27–112	0	Newala Hospital, mild
70*r*		9	5. iii. 53	S 27–112	> 2·3	illness
19*v*	Twikali d/o	3 hr.	22. ii. 53	S 27–112	0·3	Namikupa. Rash on
89*r*	Mwache	11	6. iii. 53	S 27–112	> 2·3	back when first seen
20*v*	Fatu d/o	2	22. ii. 53	S 27–112	0·3	Namikupa
90	Mangororo	14	6. iii. 53	S 27–112	> 2·3	
21*v*	Josephina d/o	1½	22. ii. 53	S 27–112	0·3	Tandahimba
92	Mniwasa	14	6. iii. 53	S 27–112	> 2·3	
22*v*	Stella d/o Mbepo	4	22. ii. 53	S 27–112	0·3	Tandahimba, sister of
94		16	6. iii. 53	S 27–112	> 2·3	the following
23*v*	Joi d/o Mbepo	2	22. ii. 53	S 27–112	0·3	Tandahimba, oral
93*r*		14	6. iii. 53	S 27–112	> 2·3	temp. 103·6° F.
25*v*	Sabiyao s/o	16 hr.	23. ii. 53	S 27–112	< 0·8	Mkonjowano, severe
101	Nachuli	16	9. iii. 53	S 27–112	> 2·8	case, very sore joints
27*v*	Joi Athumani	5 hr.	22. ii. 53	S 27–102	0	Liteho, host of a type
100		15	9. iii. 53	S 27–102	2·1	strain
29*v*	Yohana s/o	1	24. ii. 53	S 27–112	1·0	Tandahimba
91*r*	Kanole	11	6. iii. 53	S 27–112	> 2·3	
32*v*	Dunstain s/o	1	25. ii. 53	S 32–15A	0	Muhidya, host of a
95*r*	Nakulya	10	6. iii. 53	S 32–15A	2·5	type strain
42	Jamima d/o	2½	2. iii. 53	S 27–112	0·3	Mkoo, donor covered
109	Serekali	11	10. iii. 53	S 27–112	> 2·3	with typical rash
43	Naomi d/o	3½	2. iii. 53	S 27–112	0·3	Mkoo
108	Athumani	11	10. iii. 53	S 27–112	> 2·3	
46*v*	Esha d/o Asmani	1½	3. iii. 53	S 27–112	0·8	Kitama
104		8	9. iii. 53	S 27–112	2·3	

Notes. N.I. = Neutralization index.
a = seen at first bleeding by African dresser only.
v = virus probably present in original specimen.
r = Chikungunya virus re-isolated from specimen after 2½ years at − 20° C.

Table 6. *Neutralization indices and neutralization titres of sera of the Lindi Medical Department African Staff*

Serum no.	Name	N.I.	N.T.	Remarks
S 1	Gervase Fidelis	2·8	1/6	Febrile attack
S 110		3·0	1/192	
S 2	Sulemani Kipande	3·5	—	—
—	—	—	—	—
S 3	Luis Mwambe	2·7	1/10	Febrile attack
S 112	—	2·8	1/192	—
S 4	Dunford Kalindaya	1·4	—	Febrile attack
S 111	—	2·8	—	Joint pains
S 8	John Longole	0	—	Febrile attack
S 113	—	0	—	(Chronic malarious subject)

N.T. = Neutralization titres, i.e. the dilutions of serum protecting half the mice when inoculated mixed with about 100 LD_{50} of virus.

virus in the weeks following inoculation. A rhesus monkey, MR 687, was accidentally discovered to have had antibody to this virus in its serum; this animal had undergone no experimentation up to that time beyond periodic bleeding and a period as a sentinel monkey at Zika some years before, during which it had been returned febrile to Entebbe.

Table 7. *Neutralization indices on sera collected from selected groups of convalescents*

(*a*) A group of sera taken from the earliest cases reported on the central plateau

Cases in Lihombo infected during August 1952				Cases in Makukwe infected during September 1952			
Serum no.	Case no.	Name	N.I.	Serum no.	Case no.	Name	N.I.
S 60	1	Isumail s/o Salimu	3·1	S 54	9	Mwidachi s/o Hamisi	3·2
S 61	4	Daima s/o Mwenyemkuu	2·5	S 55	—	Jirani s/o Mshamu	3·6
S 62	5	Mtipa s/o Soloko	2·5	S 56	—	Fatu d/o Saidi	3·6
S 63	3	Mwamulu d/o Nimaika	2·5	S 57	—	Sawabu d/o Mwadachi	4·5
S 64	7	Vanango d/o Isumail	3·0	S 58	—	Pasa d/o Chipote	2·8
S 65	8	Alangule d/o Nipa	2·8	S 59	—	Sawabu d/o Salimu	2·5

Note. The case numbers give the reported chromological sequence.

(*b*) A group of sera from Newala schoolgirls who suffered attacks of Chikungunya in December 1952

Serum no.	Name	N.I.	Serum no.	Name	N.I.
S 71	Julia Hemedi	3·1	S 79	Damaris Katanle	4·6
S 72	Agatha Saidi	3·9	S 82	Veronica Malyalya	2·1
S 73	Agatha Nayopa	4·0	S 83	May Muyahe	3·1
S 74	Emily Masango	1·3	S 84	Agatha Salimu	1·4
S 75	Agnes Zuberi	3·7	S 85	Elizabeth Hasani	3·1
S 76	Lois Chinahanlika	0·8	S 86	Harriet Mwapachi	1·3
S 77	Eme Minyope	3·9	S 87	Damaris Lihonda	3·1
S 78	Joy Mchauru	3·3	S 88	Veronica Mshamu	2·0

(*c*) Sera from two more convalescents

Serum no.	Date taken	Name	Days after onset	N.I.	Remarks
S 6	20. ii. 53	Dr Amin	22	2·7	Severe case contracted after 3 hr. visit to Newala, 3 day incubation
S 9	22. ii. 53	Zainabu d/o Hatibu	10	1·6	Convalescent typical case with severe joint pains as sequelae

VII. DISCUSSION

The strains of Chikungunya virus studied were isolated from human sera, a captive mosquito which had fed on a patient, and a glycerinated pool of some of the *A. aegypti* captured in Newala. In addition, the deaths of the majority of baby mice in the groups inoculated with other materials after an appropriate inoculation period showed that the virus was probably present in at least seven sera, in four mosquitoes and in a pool of glycerinated culex mosquitoes. The virus was apparently very abundant in Newala at the time of the investigation. Antibodies in the

sera of human convalescents were demonstrated; notably in fifteen pairs of sera, in which antibody was first found in the early convalescent samples, showing that it had first appeared shortly after the illness. Neutralizing antibody was also shown to be present in the sera of cases infected several months before and in the sera of monkeys inoculated with human sera and wild mosquitoes.

The evidence linking the virus isolated to the human disease is very strong, and it is for that reason that the native name is attached to it. The geographical title is not applied because the illness caused by the virus is a clinical variant of classical dengue differing in the absence of headache, of tenderness on pressure to the eyeballs, and of pain on eye movements. The joint pains may be of crippling severity, meriting the old term 'break-bone fever', a name which is already associated with dengue and therefore could not be used here. Most viruses called after place-names produce encephalitides which are indistinguishable clinically, and for which there is no special term. A place name is initially a useful label, but many viruses so labelled are found on later study to have such a wide distribution that the name is misleading.

Chikungunya is a descriptive term, which can be roughly translated as the 'disease that bends up the joints'; it has already appeared in reports (*Virus Research Institute Annual Report*, 1953; Lumsden, 1955; Robinson, 1955) and is current in a district with about 140,000 inhabitants. It is most probable that the associated virus will eventually be regarded as a type of dengue.

The significance of Makonde virus is not clear and a further report will be devoted to it.

Thanks are due to Dr Robinson, who first observed the epidemic, was able to bring it to the notice of the Health Department of Lindi and Medical Headquarters at Dar-es-Salaam and who gave most valuable help in Newala itself. Dr Franks, of the Lindi Health Department, loaned transport, stores and trained personnel to assist in the investigation. The District Commissioner in Newala accommodated us and provided help of every kind. Dr Lumsden provided some interesting suggestions, and my other colleagues in this Institute, and in particular Dr Haddow, advised me continually. Dr Gillett took care of the captive mosquitoes and their feeding. Mr Mason re-isolated virus from many stored samples of serum and other materials. My wife assisted in the preparation of the manuscript.

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REFERENCES

LUMSDEN, W. H. R. (1955). *Trans. R. Soc. trop. Med. Hyg.* **49**, 33–57.
MEIKLEJOHN, G., ENGLAND, B. & LENNETTE, E. H. (1952). *Amer. J. trop. Med. Hyg.* **1**, 51.
REED, L. J. & MUENCH, H. (1938). *Amer. J. Hyg.* **27**, 493.
ROBINSON, M. C. (1955). *Trans. R. Soc. trop. Med. Hyg.* **49**, 28–33.
SABIN, A. B. (1952). *Amer. J. trop. Med. Hyg.* **1**, 30.
SCHLESINGER, R. W. & FRANKEL, J. W. (1952). *Amer. J. trop. Med. Hyg.* **1**, 66.
SMITHBURN, K. C. (1951). *J. Immunol.* **68**, 441.
TAYLOR, R. M. & HURLBUT, H. S. (1953). *J. Egypt. med. Ass.* **36**, 489.

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